TITLE OF INVENTION

Modulation of Cellular Activity

FIELD OF INVENTION

This invention relates to the modulation of cellular activity and particularly to the modulation of cellular activity in a human employing forms of hyaluronic acid, including specific molecular amounts and fractions of, for example, sodium hyaluronate and molecules which mimic forms of hyaluronic acid.

BACKGROUND OF INVENTION

Cell adhesion molecules, which were originally thought to be mere glue that hold cells together in tissues, are now also thought to have a wider contribution to the function of the human body. In this regard, see BIOTECH GETS A GRIP ON CELL ADHESION, SCIENCE, Volume 260 (May 14, 1993).

Novel therapies have been proposed based on these cell adhesion molecules. One class of endothelial adhesion molecules referred to includes the endothelial adhesion molecule ICAM-1. This molecule (ICAM-1) was discovered to carry the human rhino viruses (which cause about 50% of colds) into the body's cells. The rhino viruses enter the body's cells by latching on to the adhesion molecule ICAM-1.

By using soluble ICAM-1 as a decoy, it was proposed that the infection of the cells can be blocked. However, soluble ICAM just was not very sticky and therefore, the proposal has not reached the market.

Genetic engineering techniques have been used to fuse antibody fragments to rhino virus - binding portions of ICAM-1 in an attempt to discover a prevention for the common cold.

Another adhesion molecule, CD44, is found normally on lymphocytes. The CD44 adhesion molecule also studs the surface of pancreatic tumour cells that are metastatic. The CD44 molecules may have inadvertently disguised the cancer cells and allows them to circulate freely in the bloodstream.

It has now been discovered that adhesion molecule ICAM-1 is a cell-

surface receptor for hyaluronic acid (hyaluronan). ICAM-1 is expressed (produced and put on the cell surface) in the liver endothelial cells and corneal epithelium cells. ICAM-1 is overexpressed in inflammation, cancer and infection.

HARLEC (Hyaluronic Acid [Hyaluronan] Receptors Liver Endothelial Cells) is also a cell-surface receptor for Hyaluronan and is also expressed (produced and put on the cell surface) in liver endothelial cells and corneal epithelial cells. (This Inventor believes the two to be one and the same adhesion molecule or, at the very least, so intimately related to be one another as to be close to being one and the same.)

The molecules CD44 and RHAMM (Receptor for HA-Mediated Motility) are also cell-surface receptors for hyaluronic acid (hyaluronan). RHAMM is a Regulatory molecule. CD44 is an adhesion molecule.

It is therefore an object of the invention to provide new methods of treatment of disease and conditions, new uses for forms of hyaluronic acid (hyaluronan) (HA), new dosage amounts of pharmaceutical compositions and new pharmaceutical compositions suitable for use in such treatments and uses of forms of HA.

It is a further object of this invention to provide new methods of treatment of disease and conditions which employ molecules that mimic hyaluronic acid and pharmaceutically acceptable salts thereof (for example sodium hyaluronate) in respect of their ability to bind to the same receptors as the forms of hyaluronic acid (for example, high affinity cell-surface receptors for hyaluronic acid), new uses for these hyaluronic acid mimicking molecules, new dosage amounts of pharmaceutical compositions which comprise such molecules and new pharmaceutical compositions suitable for use in such treatments and uses of the mimicking molecules.

Further and other objects of the invention will be realized to those skilled in the art from the following summary of the invention and examples.

SUMMARY OF INVENTION

According to one aspect of the invention, a method for the modulation of cellular activity of tissue and cells expressing a high affinity cell-surface receptor for hyaluronic acid such as an adhesion molecule (for example, ICAM-1, HARLEC and CD44) and a regulatory molecule (for example, RHAMM) of a human is provided the method comprising the administration of a non-toxic effective amount of a form of hyaluronic acid (for example, hyaluronic acid, a pharmaceutically acceptable salt thereof, [for example, sodium hyaluronate having an average molecular weight less than 750,000 daltons [for example, less than 50,000 daltons, between about 100,000 to 150,000 daltons and 225,000 daltons] and from Hyal Pharmaceutical Corporation within the range of 150,000-225,000 daltons and those disclosed in U.S. Patent Application 08/143983, and molecular weight fractions of a form of sodium hyaluronate (for example, fractions disclosed in Canadian Letters Patent 1205031 (to Fidia) such as those from 50,000-100,000 daltons, 250,000-350,000 daltons and 500,000-730,000 daltons, or other fractions such as less than 50,000 daltons and those between 100,000-150,000 daltons, or homologues, analogues, derivatives, complexes, esters, fragments and/or sub units of hyaluronic acid and/or combinations thereof) preferably hyaluronic acid, a pharmaceutically acceptable salt thereof (for example sodium hyaluronate) and combinations thereof, having a molecular weight less than 750,000 daltons, and molecules which mimic the forms of hyaluronic acid aforesaid in respect of their ability to bind to the same receptors as the form of hyaluronic acid, to a human to modulate cellular activity of tissues and/or cells expressing a molecule such as a high affinity cell-surface receptor for hyaluronic acid (for example, an adhesion or regulatory molecule) in the human body, in a pharmaceutical carrier or excipient tolerable by the human (for example, sterile water).

Thus, for example, where an inflammatory response (reaction) may be set up in the area of damage or trauma, the response may include the migration

of inflammatory cells (for example, neutrophils, macrophages, and other white blood cells) to the area. The damage caused by setting up the inflammatory response may be greater than the actual damage or trauma caused. The same will be the case for fibrosis. The administration of the form of hyaluronic acid will bind to the HA receptor (the high affinity cell-surface receptor) of the molecule, for example adhesion molecule or regulatory molecule, thus modulating the body's response and thus, subsequent and consequent damage. In the same way, the administration of the molecules which mimic the form of hyaluronic acid in respect of their ability to bind to the same receptors (as the form of hyaluronic acid) will bind to the HA receptor. Examples of molecules which mimic the form of hyaluronic acid are for example, monoclonal antibodies which bind to the active site of the receptor, peptides which fit into the site of the receptor and synthetic chemicals which fit into the receptor site.

Drugs for the treatment of the disease and for condition may also accompany the form of hyaluronic acid and/or molecules which mimic the form of hyaluronic acid in which event the drug enhances the modulation of the form of the HA and molecule mimicking the HA activity. The form of HA further targets the drug to the adhesion molecule (for example, ICAM-1, HARLEC, and CD44) and RHAMM, a Regulatory molecule.

Suitable drugs may comprise for example, a free radical scavenger (for example ascorbic acid (Vitamin C)), an anti-cancer agent, chemotherapeutic agent, anti-viral agents for example a nonionic surfactant, e.g. nonoxynol-9 [nonylphenoxy polyethoxy ethanol] found in Delfen™ contraceptive cream, and anionic surfactants (e.g. cetyl pyridinium chloride) and cationic surfactants (e.g. benzalkonium chloride), non-steroidal anti-inflammatory drugs (NSAID) for example indomethacin, naproxen, diclofenac and (+/-) tromethamine salt of ketorolac (sold under the trademark Toadol™) and steroidal anti-inflammatory drugs, anti-fungal agent, detoxifying agents (for example for administration rectally

in an enema), analgesic, bronchodilator, anti-bacterial agent, antibiotics, drugs for the treatment of vascular ischemia (for example diabetes and Berger's disease), anti-body monoclonal agent, minoxidil for topical application for hair growth, diuretics (for example furosemide (sold under the trademark Lasix™), immunosuppressants (for example cyclosporins), lymphokynes (such as interleukin - 2 and the like), alpha-and-B-interferon, and the like.

According to another aspect of the invention, use of a form of hyaluronic acid, for example, hyaluronic acid, a salt thereof (for example, sodium hyaluronate having a molecular weight less than 750,000 daltons, for example, 225,000 daltons), molecular fractions thereof [for example, those disclosed from Hyal Pharmaceutical Corporation in the range of 150,000-225,000 daltons, those in Canadian Letters Patent 1205031 (to FIDIA) such as those from 50,000-100,000 daltons, 250,000-350,000 daltons and 500,000-730,000, daltons or other fractions], homologues, analogues, derivatives, complexes, esters, fragments and/or subunits of hyaluronic acid and/or combinations thereof and a molecule which mimics the form of hyaluronic acid in respect of binding to the same receptors as the form of hyaluronic acid is provided to modulate cellular activity of tissues and/or cells expressing a high affinity cell-surface receptor in the human body. The form of hyaluronic acid may be used with a pharmaceutically tolerable excipient or carrier (for example, sterile water). Once again, tissue or cell modulation is enhanced in a person when suffering a disease and/or condition. The HA and/or HA mimicking molecule binds to the HA receptors of the molecule such as the high affinity cell-surface receptor for a form of hyaluronic acid, for example an adhesion or regulatory molecule. Once again, the form of HA and HA mimicking molecule may be used with a suitable drug (as described previously). Both the form of HA, HA mimicking molecule and drug are in effective non-toxic amounts.

The form of HA and HA mimicking molecule may also be used to prevent a disease or condition (such as a cold) by preventing the human rhino

virus from entering the body's cells by the form of HA and/or HA mimicking molecule binding with the ICAM-1 adhesion molecules (instead of the Rhino virus binding or latching on).

Thus, according to another aspect of the invention, Applicant provides a novel method of preventing a disease and/or condition, the method comprising administering an effective non-toxic amount of a form of hyaluronic acid (for example, hyaluronic acid, a pharmaceutically acceptable salt thereof, [for example, sodium hyaluronate having a molecular weight less than 750,000 daltons for example, 225,000 daltons, from Hyal Pharmaceutical Corporation in the range of 150,000-225,000 daltons and those disclosed in U.S. Patent Application 08/143983 and those molecular weight fractions of a form of sodium hyaluronate [for example, fractions disclosed in Canadian Letters Patent 125031 (to Fidia) such as those from 50,000-100,000 daltons, 250,000-350,000 daltons and 500,000-730,000 daltons or other fractions], homologues, analogues, derivatives, complexes, esters, fragments and/or subunits of hyaluronic acid and/or combinations thereof and/or molecules which mimic the forms of hyaluronic acid in respect of their ability to bind to the same receptors as the form of hyaluronic acid, to a human to modulate cellular activity of tissues and/or cells expressing a molecule such as a high affinity cell-surface receptor for a form of hyaluronic acid, for example, an adhesion or regulatory molecule in the human body to thereby prevent a disease or condition. The administration of the form of HA or mimicking molecule is preferably in a pharmaceutically tolerable excipient carrier (for example, sterile water). Thus, for example, the common cold may be prevented. Drugs which may assist to prevent a disease or condition may be administered with the form of hyaluronic acid (HA) and/or HA mimicking molecule. Thus, for example, acetylsalicylic acid may be administered with a form of HA and/or mimicking molecule for the prevention of stroke.

The administration of the form of hyaluronic acid (HA) and HA

mimicking molecule thus for example inhibits cellular adhesion and/or modulates cellular activity in a human. The HA and mimicking molecule bind to the HA receptors of the molecule at the high affinity cell-surface receptor for a form of the hyaluronic acid, for example, adhesion molecule (for example ICAM-1, HARLEC and CD44) and/or regulatory molecule (Rhamm, for example). Thus, the administration of the form of hyaluronic acid and mimicking molecule will bind to the HA receptor of the molecule, thus preventing the disease or condition (for example, preventing the binding of the rhino virus to the adhesion molecule ICAM-1 thereby preventing the rhino virus entering the body's cells by latching onto the adhesion molecule ICAM-1).

Where drugs are taken for the prevention of a disease and/or condition may, as discussed above, accompany the form of hyaluronic acid used to prevent the disease or condition. For example, for the prevention of a stroke, people today take acetylsalicylic acid (aspirin) in an effective amount. The drug may be accompanied by a suitable form of hyaluronic acid in an effective non-toxic amount (for example sodium hyaluronate having a molecular weight less than 750,000 daltons) and/or by a mimicking molecule in an effective amount. Thus, the taking of the two together will, it is believed, reduce the risk of stroke even more than the individual taking aspirin alone. The form of HA further targets the drug to the areas in need of treatment while the form of HA binds with the high affinity cell-surface receptor for a form of hyaluronic acid as expressed by cells and/or tissue such as an adhesion molecule (for example, ICAM-1, HARLEC and CD44) and a Regulatory molecule (for example RHAMM) modulating their activity.

The administration may be given, among other methods, intravenously, intra-arterially, intraperitoneally, intrapleurally, percutaneously, (intra-cutaneously - into the skin (for example, targeting the epidermis), by topical application of effective amounts and by application to the mucosa by topical application, for example, intranasally, rectally (for example, in the form of an

enema or by topical application of specific areas in the rectum), and by direct injection.

Where the form of hyaluronic acid is to be administered, varying effective doses may be employed - for example, 10 to 1000mg/70kg. person with optimal doses tending to range between 50 and 500mg/70kg. person. As there is no toxicity in humans, the hyaluronic acid can obviously be administered in a dose excess (for example 3000mg/70kg. individual) without any adverse effects.

Where the administration is topical (for example, applied to the skin or mucosa), in excess of 5 mg per cm^2 (square centimetre) of skin or exposed tissue (including mucosa) of the form of hyaluronic acid, should be applied.

With respect to the treatment of pain, preferably a minimum of 10mg per cm^2 (10mg/cm^2) of the form of HA should be applied topically.

The HA mimicking molecules can be given in effective dosage amounts as persons skilled in the art may use.

Thus, effective dosage amounts can contain at least about 10mg of the form of hyaluronic acid per 70kg person to in excess of 1000mg per 70kg person where the administration is non-topical. When an NSAID for example, indomethacin (dissolved in n-methyl glucamine) or other NSAID is administered with greater than 200mg hyaluronic acid for example, sodium hyaluronate per 70kg person with 1 - 2 mg/kg body weight of the person, of the NSAID (in one instance indomethacin and NMG), no major toxic side effects occur such as gastro-intestinal distress, neurological abnormalities, depression, etc., even at elevated amounts of indomethacin (if necessary). If the amount of hyaluronic acid is decreased below that amount, the usual side effects may begin to reoccur. Where administration is topical, the dosage amounts contain at least about 5mg of the form of hyaluronic acid / cm^2 of the skin, mucosa or tissue to which the dosage amount is to be applied.

The Drugs accompanying the form of hyaluronic acid and/or HA

mimicking molecule are adjusted with the form of treatment to be an effective non-toxic amount thereof.

One form of hyaluronic acid and/or pharmaceutically acceptable salts thereof (for example sodium salt) and homologues, analogues, derivatives, complexes, esters, fragments, and sub units of hyaluronic acid, preferably hyaluronic acid and salts and thereof suitable for use with Applicant's invention is supplied by Hyal Pharmaceutical Corporation. One such form is a 15 ml vial of Sodium hyaluronate 20mg/ml (300mg/vial - Lot 2F3). The sodium hyaluronate is a 2% solution with a mean average molecular weight of about 225,000 daltons. The vial also contains water q.s. which is triple distilled and sterile in accordance with the U.S.P. for injection formulations. The vials of hyaluronic acid and/or salts thereof may be carried in a Type 1 borosilicate glass vial closed by a butyl stopper which does not react with the contents of the vial.

The fraction/amount of hyaluronic acid and/or pharmaceutically acceptable salts thereof (for example sodium salt) and homologues, analogues, derivatives, complexes, esters, fragments, and sub units of hyaluronic acid, preferably hyaluronic acid and pharmaceutically acceptable salts thereof may comprise hyaluronic acid and/or salts thereof having the following characteristics:

a purified, substantially pyrogen-free fraction of hyaluronic acid obtained from a natural source having at least one characteristic selected from the group consisting of the following:

i) a molecular weight within the range of 150,000-225,000;

ii) less than about 1.25% sulphated mucopolysaccharides on a total weight basis;

iii) less than about 0.6% protein on a total weight basis;

iv) less than about 150 ppm iron on a total weight basis;

v) less than about 15 ppm lead on a total weight basis;

vi) less than 0.0025% glucosamine;

vii) less than 0.025% glucuronic acid;

viii) less than 0.025% N-acetylglucosamine;

ix) less than 0.0025% amino acids;

x) a UV extinction coefficient at 257 nm of less than about 0.275;

xi) a UV extinction coefficient at 280 nm of less than about 0.25; and

xii) a pH within the range of 7.3-7.9.

Preferably the hyaluronic acid is mixed with water and the fraction of hyaluronic acid fraction has a mean average molecular weight within the range of 150,000-225,000. More preferably the fraction of hyaluronic acid comprises at least one characteristic selected from the group consisting of the following characteristic.

i) less than about 1% sulphated mucopolysaccharides on a total weight basis;

ii) less than about 0.4% protein on a total weight basis;

iii) less than about 100 ppm iron on a total weight basis;

iv) less than about 10 ppm lead on a total weight basis;

v) less than 0.00166% glucosamine;

vi) less than 0.0166% glucuronic acid;

vii) less than 0.0166% N-acetylglucosamine;

viii) less than 0.00166% amino acids;

ix) a UV extinction coefficient at 257 nm of less than about 0.23;

x) a UV extinction coefficient at 280 nm of less than 0.19; and

xi) a pH within the range of 7.5-7.7

In addition, Applicant proposes successful employment of sodium hyaluronate produced and supplied by LifeCore™ Biodmedical, Inc. having the following specifications:

Characteristics	Specification
Appearance	White to cream colored particles
Odor	No perceptible odor
Viscosity Average Molecular Weight	< 750,000 Daltons
UV/Vis Scan, 190-820nm	Matches reference scan
OD, 260 nm	< 0.25 OD units
Hyaluronidase Sensitivity	Positive response
IR Scan	Matches reference
pH, 10mg/g solution	6.2-7.8
Water	8% maximum
Protein	< 0.3 mcg/mg NaHy
Acetate	< 10.0 mcg/mg NaHy
Heavy Metals, maximum ppm	

As	Cd	Cr	Co	Cu	Fe	Pb	Hg	Ni
2.0	5.0	5.0	10.0	10.0	25.0	10.0	10.0	5.0

Microbial Bioburden	None observed
Endotoxin	< 0.07EU/mg NaHy
Biological Safety Testing	Passes Rabbit Ocular Toxicity Test

Another form of sodium hyaluronate is sold under the name Hyaluronan HA-M5070 by Skymart Enterprises, Inc. having the following specifications:

Specification's Test

Results

Lot No.	HG1004
pH	6.12

Condroitin Sulfate	not detected
Protein	0.05%
Heavy Metals	Not more than 20 ppm
Arsenic	Not more than 2 ppm
Loss on Drying	2.07%
Residue on Ignition	16.69%
Intrinsic Viscosity	12.75 dl/s (XW: 679,000)
Nitrogen	3.14%
Assay	104.1%
Microbiological Counts	80/g
E. coli	Negative
Mold and Yeast	Not more than 50/g

Other forms of hyaluronic acid and/or its salts, and homologues, derivatives, complexes, esters, fragments and sub units of hyaluronic acid may be chosen from other suppliers, for example those described in the prior art. The following references teach hyaluronic acid, sources thereof and processes of the manufacture and recovery thereof.

United States Patent 4,141,973 teaches hyaluronic acid fractions (including sodium salts) having:

"(a) an average molecular weight greater than about 750,000, preferably greater than about 1,200,00 - that is, a limiting viscosity number greater than about 1400 $cm^3/g.$, and preferably greater than about 2000 $cm^3/g.$;

(b) a protein content of less than 0.5% by weight;

(c) ultraviolet light absorbance of a 1% solution of sodium hyaluronate of less than 3.0 at 257 nanometers wavelength and less than 2.0 at 280 nanometers wavelength;

(d) a kinematic viscosity of a 1% solution of sodium hyaluronate in

physiological buffer greater than about 1000 centistokes, preferably greater than 10,000 centistokes;

(e) a molar optical rotation of a 0.1 - 0.2% sodium hyaluronate solution in physiological buffer of less than -11 x 10^3 degree - cm^2/mole (of disaccharide) measured at 220 nanometers;

(f) no significant cellular infiltration of the vitreous and anterior chamber, no flare in the aqueous humor, no haze or flare in the vitreous and no pathological changes to the cornea, lens, iris, retina, and chorold of the owl monkey eye when one milliliter of a 1% solution of sodium hyaluronate dissolved in physiological buffer is implanted in the vitreous replacing approximately one-half the existing liquid vitreous, said HUA being

(g) sterile and pyrogen free and

(h) non-antigenic."

Canadian Letters Patent 1,205,031 (which refers to United States Patent 4,141,937 as prior art) refers to hyaluronic acid fractions having average molecular weights of from 50,000 to 100,000; 250,000 to 350,000; and 500,000 to 730,000 and discusses processes of their manufacture.

Where high molecular weight hyaluronic acid (or salts or other forms thereof) is used, it must be diluted to permit administration and ensure no intramuscular coagulation.

According to one aspect of the invention, a dosage amount of a pharmaceutical composition is provided for the modulation of cellular activity of tissue and cells expressing a molecule having a high affinity cell-surface receptor for a form of hyaluronic acid, for example, an adhesion molecule (for example, ICAM-1, HARLEC and CD44) and a Regulatory molecule (for example, RHAMM) of a human, the dosage amount comprising a non-toxic effective amount of a form of hyaluronic acid (for example, hyaluronic acid, a pharmaceutically acceptable salt

thereof, [for example, sodium hyaluronate having a molecular weight less than 750,000 daltons for example, 225,000 daltons] , molecular weight fractions of a form of sodium hyaluronate (for example, fractions from Hyal Pharmaceutical Corporation in the range of 150,000-225,000 daltons, and those disclosed in U.S. Application 08/143983 and those disclosed in Canadian Letters Patent 1205031 (to Fidia) such as those from 50,000-100,000 daltons, 250,000-350,000 daltons and 500,000-730,000 daltons, or other fractions such as less than 50,000 daltons and those between about 100,000-150,000 daltons, homologues, analogues, derivatives, complexes, esters, fragments and/or sub units of hyaluronic acid and/or combinations thereof) and HA mimicking molecules in respect of their ability to bind to the same receptors, to a human to modulate cellular activity of tissues and/or cells expressing a molecule having a high affinity cell-surface receptor for a form of hyaluronic acid, for example an adhesion molecule and/or a Regulatory molecule in the human body, in a pharmaceutical carrier excipient tolerable by the human (for example, sterile water).

The result of the treatment by the dosage amount is that tissue and/or cell modulation is enhanced in the person suffering the disease or condition. The result of the administration of the form of hyaluronic acid (HA) or HA mimicking molecule is, for example, the inhibition of cellular adhesion and/or modulation and/or regulation of cellular activity. The HA and HA mimicking molecule each binds to the HA receptors of the molecule (for example, adhesion molecule, Regulatory molecule and the like). Thus prevention of a disease or condition may be accomplished. The unexpected high affinity of the HA and HA mimicking molecule for the cell-surface HA receptors enable the modulation of disease or condition for example, the modulation of the inflammatory process, fibrosis, and, for oncogene control (to prevent cancer and metastases).

The dosage amount of the pharmaceutical composition may also comprise an effective non-toxic amount of a medicine (drug) or therapeutic agent

for the treatment of the disease and/or condition accompanying the form of hyaluronic acid or form of hyaluronic acid mimicking molecule for the prevention of the disease or condition. The drug therefore can enhance the modulation of the form of the HA and HA mimicking molecule activity. The form of HA further targets the drug to the cell-surface receptor of the molecule, such as for example, ICAM-1, HARLEC, and CD44 (examples of adhesion molecules) and RHAMM (a Regulatory molecule).

Thus, according to another aspect of the invention, a dosage amount of a pharmaceutical composition may comprise:

i) a medicinal and/or therapeutic agent in a non-toxic therapeutically effective amount to treat a disease or condition;

ii) a non-toxic therapeutically effective amount of a form of hyaluronic acid (for example, hyaluronic acid, a pharmaceutically acceptable salt thereof, [for example, sodium hyaluronate having a molecular weight less than 750,000 daltons, for example, 225,000 daltons], molecular weights of a form of sodium hyaluronate (for example, from Hyal Pharmaceutical Corporation within the range of 150,000-225,000 daltons and those disclosed in U.S. Patent Application 08/143983 and those disclosed in Canadian Letters Patent 1205031 (to Fidia) such as those from 50,000-100,000 daltons, 250,000-350,000 daltons, and 500,000-730,000 daltons, or other fractions, such as less than 50,000 daltons and between 100,000-150,000 daltons, homologues, analogues, derivatives, complexes, esters, fragments and/or sub units of hyaluronic acid and/or

combinations thereof) and HA mimicking molecules which mimic the form of hyaluronic acid in respect of their ability to bind to the same receptors, to a human to modulate cellular activity of tissues and/or cells expressing a molecule having a high affinity cell-surface receptor for a form of hyaluronic acid, for example, an adhesion molecule or regulatory molecule in the human body, and

iii) a pharmaceutically tolerable excipient (for example, sterile water);

wherein component (ii) is in such form that when the dosage amount of the pharmaceutical composition is administered/applied, component (ii) is available to modulate the cellular activity of tissue and cells expressing, for example, an adhesion molecule (for example, ICAM-1, HARLEC and CD44) and a Regulatory molecule (RHAMM) of a human by for example, binding with the receptor for the form of hyaluronic acid and/or HA mimicking molecules, and component (ii) is immediately available to transport component (i) in the body or into the skin as the case may be if component (ii) is a form of hyaluronic acid.

Suitable medicines (drugs) and therapeutic agents may comprise for example, a free radical scavenger (for example ascorbic acid (Vitamin C)), an anti-cancer agent, chemotherapeutic agent, anti-viral agents for example a nonionic surfactant, e.g. nonoxynol-9 [nonylphenoxy polyethoxy ethanol] found in Delfen™ contraceptive cream, and anionic surfactants (e.g. cetyl pyridinium chloride) and cationic surfactants (e.g. benzalkonium chloride), non-steroidal anti-inflammatory drugs (NSAID) for example indomethacin, naproxen and (+/-) tromethamine salt of ketorolac (sold under the trademark Toadol™) and steroidal anti-inflammatory drugs, anti-fungal agent, detoxifying agents (for example for administration rectally in an enema), analgesic, bronchodilator, anti-bacterial agent, antibiotics, drugs for

the treatment of vascular ischemia (for example diabetes and Berger's disease), anti-body monoclonal agent, minoxidil for topical application for hair growth, diuretics (for example furosemide (sold under the trademark Lasix™), immunosuppressants (for example cyclosporins), lymphokynes (such as interleukin - 2 and the like), alpha-and-B-interferon, and the like.

Dosage amounts of the pharmaceutical compositions may be accumulated in containers to supply multiple dosage amounts from which an individual dosage amount may be taken.

The invention is demonstrated with respect to the following examples:

Example 1

The polysaccharide hyaluronan (hyaluronic acid; HA or HYA) is rapidly cleared from the circulation, primarily by endothelial cells of the liver via receptor-mediated endocytosis. The receptor for HYA on these cells has been characterized and purified from rat liver endothelial cells (LEC) and polyclonal antibodies produced. Other cell-surface "receptors" for HYA have been described and include the lymphocyte homing receptor CD44 and a receptor for HYA-mediated motility of fibroblasts (RHAMM). Many tumours have been reported to be enriched in HYA and HYA binding sites on cells derived from some tumours have been described.. Previous findings have shown that transformed tissues such as in mouse mastocytomas and human mammary carcinomas stain positively for the HA-receptor originally found on liver endothelial cells.

The present work was initiated in order to determine if accessible HYA binding sites are present in tumour tissue *in vivo*, and the relation of these possible sites to previously described HYA-binding proteins. A recently developed ^{125}I-labelling method, that does not significantly alter the Mw of the polysaccharide nor its receptor-binding properties, was used.

MATERIALS AND METHODS

Polysaccharides

HA used for labelling and uptake and turnover-studies was extracted from avian tissue and supplied by Hyal Pharmaceutical Corporation, Toronto, Canada. The molecular weight distribution of the HA was determined by chromatography on a calibrated column of Sephacryl HR with porosities noted as 400, 1000 and 2000 (Pharmacia, Uppsala, Sweden) in 0.25M NaCl, 0.05% chlorbutanol. The HA content in each fraction was monitored by determination of the absorbance at 214 nm. Radioactivity was measured by gamma-counting on a Packard auto-gamma gamma-counter. (The mean average molecular weight is in the order of 450,000 daltons and (made up from powder whose mean average molecular weight is 500,000-800,000 daltons).

Labelling of HA

The HA was labelled with DL-tyrosine (Sigma chemical company St Louis, U.S.A.) as previously described after CNBr-activation of the polysaccharide by the method of Glabe et al [Glabe CG, Harty P.K. and Rosen S.D., Preparation and properties of fluorescent polysaccharides. Anal. Biochem. 130:287-294 (1983). Briefly, 15 mg HA was activated at pH 11 by 8 mg CNBr for 5 min. The activated polysaccharide was separated from the reaction mixture on a small column of Sephadex G25 (PD 10, Pharmacia, Uppsala, Sweden) equilibrated with 0.2 M borate buffer pH 8.0. The activated HA was incubated over night with 1 mg tyrosine (T). The T bound to HYA (T-HA) was separated from unbound T on a PD 10 column equilibrated with phosphate buffered saline (pH 7,5)(PBS), containing NaCl (8g/l), KCl (0,2 g/l), KH_2PO_4 (0,2g/l) and Na_2HPO_4 (1,15 g/l).

A part of the T-HA was iodinated with ^{125}I by placing 100µg of T-HA

together with 0.5 mCi ^{125}I in a small glass tube covered with a film of 10µg 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril (Sigma chemical company, St. Louis, U.S.A.). Unincorporated ^{125}I was removed on a PD 10 column equilibrated with PBS and the iodinated T-HA (^{125}I-T-HA) stored at 5°C. The specific radioactivity was usually 1500-5000 dpm/ng.

The ^{125}I-T-HA kept a higher molecular weight-profile upon gel filtration chromatography and was found to be cleared from the circulation with the kinetics and organ distribution reported for biosynthetically labeled HA of high Mw. The ^{125}I-labelled polysaccharide was also taken up by isolated rat liver endothelial cells both *in vivo* and *in vitro*, indicating that the labelling does not interfere with the binding to specific cell-surface receptors found on these cells (1,2).

Immunostaining

All staining was made with the ABC-elite method (Vectastain^R Elite ABC). Frozen sections of 6µm were prepared and mounted on glass slides, coated with gelatin-kromalun, half a gram of kromalun ($KCr(SO_4)_2 \times 12\ H_2O$) in 750 ml aq.dest, and 5g gelatin in 250 ml aq.dest. were, after heating to 56°C, mixed. To remove unspecific binding to serum proteins, the antiserum as well as the preimmune serum was adsorbed on a Sepharose^R 4B gel (Pharmacia LKB Technology, Uppsala) coupled with rat serum proteins (6 mg protein/ml gel). Coupling was performed as described by the manufacturer (Affinity Chromatography; principles & methods, by Pharmacia LKB Biotechnology). The serum and the gel were mixed in equal volumes and incubated for 6h at 4°C. The frozen sections were fixed in cold methanol for 10 minutes, dried for 10 minutes before washing in phosphate buffered saline (PBS). Peroxidase was blocked in 0.3% H_2O_2 in methanol, and the sections were once more washed in PBS. To

block endogenous biotin and biotin-binding activity, an avidin/biotin blocking kit from Vector laboratories was used. The sections were then incubated for 30 min in PBS containing 4% goat serum (Vectastain® Elite ABC). The HARLEC antiserum, was diluted 1:300 in 4% goat serum in PBS, and incubated for one hour. After washing in PBS, the sections were incubated with the second goat anti-rabbit antibody (Vectastain® Elite ABC) diluted 1:200 in PBS for 30 min. After incubation with the second antibody the sections were washed and incubated with the ABC-Elite-complex (Vectastain® Elite ABC). To develop the colour, 10mg 3-amino-9-ethyl-carbazol in 6ml dimethylsulphoxide (DMSO) was mixed with 45ml 15mM acetate buffer pH 5 and

4 µl Perhydrol (Merck), and the sections were incubated in the mixture for 7.5 minutes. After washing, the sections were counterstained in Mayers hematoxylin for 1.5 minutes. The glass slides were mounted in Kelsers glycerol-gelatin (Merck).

Hyaluronidase treatment of sections

After methanol fixation and washing in PBS the sections were incubated with 5 U/ml of streptomyces hyaluronidase (Amano Pharmaceutical Co., Ltd Japan.), 1mg/ml pepstatin (Sigma), 0.1 M N-Ethylmaleinimid( ), 0.1M EDTA (Merck) and 5 KIE/ml Trasylol (Bayer) for two hours at 37°C. The regular staining protocol was then followed.

Uptake studies in vivo

Four female nude (Nu/Nu) rats, weighing 200-250g, were inoculated subcutaneously with approximately $3x10^6$ cells (in 0.5ml RPMI medium) of the human cellline CCL 218 (American Tissue Type Collection) in one hind leg under ether anasthesia, 14-30d before the experiments. The animals were anesthetized with pentobarbital (45 mg/kg body weight) and received an injection in the tail vein

or directly in the approximately 1-2 cm^2 large tumour of 3.4-1500µg ^{125}I-T-HYA (5-15x10^6 cpm). To reduce the specific activity and to achieve a higher chemical amount of HA, sometimes unlabelled HA in 0.05-1.0 ml 0.15 M NaCl, 10mM NaH_2PO_4, pH 7.4 was added.

After the study the animals were killed and tumour and organs assayed for radioactivity. The data were processed using a Macintosh SE/30® or a Macintosh IIsi® computer (Apple computer Inc. Cupertino, CA, U.S.A.) the graphs were constructed using the Cricket Graph® program (version 1.3, Cricket software, Malvern, PA, U.S.A.) and Canvas (version 3.0.2., Deneba Systems Inc, Miami, Fl, U.S.A.).

Scintigraphic studies

The rats were anesthetized and injected as described above. In dynamic studies the injections were made with the rats placed on the standard medium resolution collimator of the gamma-camera. Images were collected in a 64x64 pixel matrix in word mode with a 34 keV 80% window setting in a Gamma 11 system (Philips). The dynamic sequence was preset at one image per minute for 15 min. After 15 min, static images were collected at different times after injection. Images were then transferred to the Hermes® system (Nuclear Diagnostics, Hägersten, Sweden and London, U.K.) and regions of interest (ROI:6) were drawn. It was in some instances necessary to draw a ROI covering an area larger than the tumour or liver itself due to scattering.

RESULTS

By immunohistochemistry it was found that polyclonal antibodies against HARLEC recognize structures in tumours of the human coloncancer-cellline CCL 218 in nude rats. The staining was mainly localized to vessel endothelium

and over tumour cells, where it seemed to increase towards the margin of the tumour (Fig 1a). Preimmune antibodies showed virtually no staining (Fig 1b), indicating that the HARLEC-staining is specific in this system.

The invention will now be illustrated with reference to the drawings in which:

Figure 1 a) shows immunohistochemical staining of CCI 218 tumour using polyclonal antibodies to HA receptor HARLEC, b) shows the control using preimmune antibodies. See Materials and Methods for details.

When tracer doses of the ^{125}I-T-HA was injected intravenously the radioactivity disappeared rapidly from the blood to the liver and little radioactivity was left in the blood after a few minutes (Fig 2).

Figure 2 relates to disappearance of radioactivity from blood after intravenous injection in rats of 5 µg ^{125}I-T-HA. Inset: The recovery of radioactivity in different organs of the rat 10 min after an intravenous injection of 5 µg ^{125}I-T-HA. KC denotes Kupffer cells, PC denotes parenchymal cells and LEC denotes liver endothelial cells.

As the binding-capacity of the liver is so great, not much of the tracer was left in the blood after passage of the liver and only minor amounts could reach other tissues. Therefore, amounts exceeding the binding-capacity of the liver were injected and at a dose of 1.5mg HA there was still material in the general circulation at 24h and an accumulation of radioactivity in the tumours could be seen by scintigraphy (Fig 3). The major part of radioactivity was seen over the liver, but significant activity above background was observed over the tumour (Fig 3 a). The activity over the tumour was severalfold higher than what could be expected from merely increased size of the tissue, and shielding of the liver with lead produced scintigraphic images where the 50% maximum level corresponded well with the size of the tumour (Fig 3 b).

Figure 3 depicts scintigraphic images of a nude rat 3 weeks after

inoculation with the human coloncancer CCL 218 in one leg, and 24h after injection of 1.5 mg HA containing 2.5 MBq ^{125}I-T-HA. In 3b the liver is shielded by lead. Arrow points at tumor.

Immunohistochemical analysis of treated animals showed that hyaluronidase treatment of the sections caused a specific increase in HARLEC immunoreactivity (Figs. 4 a and b) without increasing the preimmune response (Figs. 4 c and d). The receptor-staining to a large extent colocalizes with the staining for HA itself (Figs. 4 e and f). The staining for HA was most prominent in the marginal zone and in and around vessels, but also other receptor-stained structures were positive for HA.

Figure 4 relates to:

a) Immunohistochemical staining of a CCl 218 tumour, after intravenous injection of ^{125}I-T-HA, using polyclonal antibodies to the HA receptor HARLEC.

b) Immunohistochemical staining as in 4 a after hyaluronidase treatment.

c) and d) As 4 a and b but with preimmune (control) antibodies.

e) and f) HA-staining of the same tumour as in 4 a-d using biotinylated HA binding protein.

As a means of reducing uptake in the liver and trying to achieve a therapeutic response, two animals received single intratumoural injections of ^{125}I-T-HA. One rat received 3.4 µg labelled HA of high specific activity (0.25MBq/µg) that to more than 90% stayed in the tumour. Small amounts reached the general circulation and was taken up by the liver (Fig 5 a). Still 15 d after injection the ratio of radioactivity over tumour and liver was similar to that observed directly after injection (Fig 5 b). The animal responded with regression of the tumour from approximately 2cm^2 to 1 cm^2 over 7d. There was a gradual decrease and softening of the tumour until it at d14 not could be sensed by palpation. Histochemical analysis after sacrifice at d15 showed that the tumour was still present with a volume of approximately 0.5 cm^2. One rat receiving only 0.025MBq

but 250 µg HA intratumourally did not respond and had at sacrifice a tumour of approximately 5cm^2 (the rats were killed at the same time).

Figure 5 depicts a scintigraphic image of a nude rat carrying a CCl 218 derived tumour that 1d (a) and 15d(b) prior to the image received 3.5µg ^{125}I-T-HA (0.75MBq) intratumourally.

Figure 6 relates to the inhibition of the cell association of ^{125}I-T-HA to LEC in culture at 37°C. Results are mean±SD, n=3 (control medium) and n=6 (1A29 medium). See Materials and Methods for details.

Figure 7 depicts the recovery of radioactivity in tumour tissue and control muscle tissue 18-20h after an intravenous injection of 2 mg ^{125}I-T-HA. Results are mean±SD, n=3. See Materials and Methods fordetails.

Figure 8 shows frozen sections of tumour tissue stained for HA and HARLEC/ICAM-1 18-20 h after an intravenous injection of 2 mg ^{125}I-T-HA. See Materials and Methods for details.

a) Staining for HA

b) Staining for HARLEC/ICAM-1

c) Staining for HARLEC/ICAM-1 after hyaluronidase treatment.

DISCUSSION

The fact that the immunohistochemical staining of HARLEC was evident in the tumours without hyaluronidase treatment (Fig 1) indicated that the binding sites are to a certain degree unoccupied, and pointed to a possible chance for uptake of circulating HA. This is in agreement with what is found for the liver receptors.

Second Example

The major site for elimination of HA (Hyaluronic acid and pharmaceutically acceptable salts thereof) from the bloodstream is via receptor mediated endocytosis by liver endothelial cells (LEC). The HA receptor (HAR)

on LEC has been characterized by HA affinity chromatography of surface ^{125}I labelled rat LEC and purified to homogeneity from rat LEC membranes. This receptor (termed HARLEC) has a mean molecular weight of approximately 90kD with a range of 85 to 100 kD and a pI of around 6.7. A monospecific polyclonal antibody against the receptor, which could inhibit binding of HA to LEC and LEC membranes, (termed anti-HARLEC) was raised. Protein species of 85-90 kD have also been detected by this antibody in kidney, spleen, thymus and lymph noes with immunoblotting. The same tissues, together with the liver, stain positively with anti-HARLEC, the immunohistochemical staining being mainly restricted to vascular regions such as the sinusoids in the liver, spleen, and lymph nodes and the capillaries in the small intestine, but also to specific structures such as thymic reticular cells. Corneal characteristics as HARLEC and are specifically stained by anti-HARLEC. The CEC staining is inhibited if the corneas are treated with HA prior to staining, while the staining intensity is restored by hyaluronidase treatment.

HARLEC was purified from while rat liver using a series of affinity chromatographic steps. In the final step, virtually all HA bound to HA-Sepharose and was specifically eluted with HA-oligosaccharides. Tryptic digestion of purified HARLEC yielded several peptides that were separated by reverse phase chromatography. Four peptides were sequenced and found to be identical to intercellular adhesion molecule-1 (ICAM-1). ICAM-1 is a glycosylated single chain protein of 80 to 114 kD with a core polypeptide of 55 kD. It is normally expressed at low levels, but has been found on normal liver endothelium at the sinusoids and on the endothelium of lymph nodes, spleen and some capillaries of the kidney, as well as on corneal endothelial cells. The immunohistochemical localization of ICAM-1 corresponds well with the staining for HARLEC that has been reported. The localization also corresponds to

tissues where HA binding and uptake have been found.

HARLEC/ICAM-1 is expressed on tumour endothelium in mouse mastocytomas and can bind to/with intravenously administered radiolabelled HA. A significant increase of radioactivity in the tumour tissue was found (approximately 5-fold relative to controls).

By immunohistochemistry it was found that the HA is localized in areas that also stain positively for ICAM-1, i.e.. mainly vessels. ICAM-1 immunoreactivity is dramatically increased after hyaluronidase treatment of the sections.

Herein, presented is further evidence that HARLEC/ICAM-1 is a receptor for HA, that HA targets also human tumours in nude rats and that the targeting is mainly via binding to HARLEC/ICAM-1 on tumour endothelium.

Materials and Methods

Polysaccharides: The HA used for labelling and uptake- and turnover studies was extracted from avian tissue and supplied by Hyal Pharmaceutical Corporation (HPC), Toronto, Canada. The mean molecular weight was approximately 450 000 Daltons.

Labelling of HA: The HA was labelled with DL-tyrosine (Sigma Chemical company St Louis, U.S.A.) and ^{125}I.

Monoclonal antibodies: The monoclonal antibody against rat ICAM-1, clone 1A29, was a kind gift of Professor M Miyasaka, Osaka University, Japan.

Cultivation of endothelial cells: Rat LEC were isolated after collagenase perfusion of rat liver and cultured without serum in RPMI medium.

Antibody inhibition experiments: 100 000-200 000 LEC cultured for 4-5h were incubated with 0.5 µg/ml ^{125}I-T-HA, and in competition experiments unlabelled HA or anti-ICAM-1 supernatant medium (diluted 1:2). As the anti ICAM-1 contained 115 ng/ml HA a control medium containing 115 ng/ml HA was made and used (diluted 1:2) as a control of inhibitory binding activity due to the HA in the antibody supernatant. The competitors were added prior to the addition of radiolabelled HA. Total incubation time was 35-40 min. After the termination of the incubations, the medium was removed, cells washed and analyzed for radioactivity as previously described(10).

Immunostaining: All staining was made with the ABC-elite method (Vectastain® Elite ABC). Frozen sections of 6µm were prepared and mounted on glass slides that were coated with gelatin-kromalun. To remove unspecific binding to serum proteins, the HARLEC antiserum was adsorbed on a Sepharose® 4B gel (Pharmacia LKB Technology, Uppsala) coupled with rat serum proteins (6 mg protein/ml gel).

The frozen sections were fixed in cold methanol for 10 minutes, dried for 10 minutes before being washed with phosphate buffered saline (PBS). Endogenous peroxidase was blocked in 0.3% H_2O_2 in methanol, and the sections were only more washed in PBS. To block endogenous biotin and biotin-binding activity, an avidin/biotin blocking kit from Vector laboratories was used. The sections were then incubated for 30 min in PBS containing 4% goat serum (Vectastain® Elite ABC). The HARLEC antiserum, was diluted 1:300 in 4% goat serum in PBS, and incubated for one hour. After washing in PBS, the sections were incubated with the secondary HRP-conjugated goat anti-rabbit antibody (Vectastain® Elite ABC) diluted 1:200 in PBS for 30 min.

The staining for HA was performed using biotinylated hyaluronan binding proteins from cartilage (b-HABP), but without CPC treatment, on frozen sections treated with methanol and blocked for endogenous biotin binding

activity with avidin/biotin blocking kit (Vector laboratories).

After incubation with the second antibody, or b-HABP, the sections were washed and incubated with the ABC-Elite-complex (Vectastain® Elite ABC). To develop the colour, peroxidase substrate kits (Vector laboratories) containing 3,3 diaminobenzidine or 3-amino-9-ethylcarbazole were used, and the sections were incubated in the mixture for 5-10 minutes. After washing, the sections were counterstained in Mayers hematoxylin for 1.5 minutes. The glass slides were mounted in Kaisers glycerol-gelatin (Merck).

Hyaluronidase treatment of sections: After methanol fixation and washing in PBS the sections were incubated with 5 U/ml of streptomyces hyaluronidase (Amano Pharmaceutical Co., Ltd Japan.), 1.8 µg/ml pepstatin (Sigma), 1.8 mM EDTA (Merck), 1.8 µg/ml soybean trypsin inhibitor (Sigma), 2.0 mM iodo acetic acid (Sigma), 0.18 mM e-amino-n-caproic acid (Sigma) and 9.0 mM benzamide (Sigma) for two hours at 37°C. The regular staining protocol was then followed.

Uptake studies in vivo: Female nude (Rowette Nu/Nu) rats, weighing approximately 200g, were inoculated subcutaneously with approximately $3x10^6$ cells (in µl s-MEM medium (Gibco)) of the human cell line CCL 218 (American Tissue Type Collection) in one hind leg, while under ether anasthesia, 14-30d before the experiments. The animals were anesthetized and received an intravenous injection of 2 mg ^{125}I-T-HYA ($2\text{-}3x10^6$ cpm). To reduce the specific activity and to achieve a higher concentration of HA, unlabelled HA in 0.15 M NaCl, 10mM NaH_2PO_4, pH 7.4 (0.05-1.0 ml) was added.

After 18-20h the animals were killed and tumour and organs assayed for radioactivity as previously described (9).

Results:

To further characterize the binding of HA to HARLEC/ICAM-1

experiments were performed with isolated rat LEC, radiolabelled HA and a well characterized monoclonal antibody towards rat ICAM-1 (Ialenti A and Di Rosa M.; Hyaluronic acid modulates acute and chronic inflammation, Agents Actions 43: 44-47). The cell association of ^{125}I-T-HA to LEC, studied at 37°C, could be inhibited by about 60% (Fig 6). However, approximately 20% of the inhibition was found to be due to the presence of HA in the antibody tissue culture medium, as indicated by the inhibition seen in control experiments using medium containing the same amount of HA but no antibodies (Fig 6).

In order to see if HARLEC/ICAM-1, expressed on endothelial cells in other tissues of the rat, has the same capacity to bind HA as LEC, turnover and tissue distribution studies were performed in rats carrying a colon carcinoma of human origin in one hind leg. It was found that 18-20h after an intravenous injection of 125I-T-HA (2 mg), most of the radioactivity was, as expected, in the liver, while very little was in the blood (not shown). The recovery of radioactivity in the tumour was more than three times the amount found in the control muscle tissue (wet weight adjusted), and this increase was statistically highly significant ($p=0.001$, $n=3$)(Fig 7).

When the tumour tissue was analyzed by histochemical techniques, it was found that the HA in the tumour was mainly localized to the stroma and vessels of the tumour (Fig 8a), and ICAM-1 in the same areas, predominantly in vessels (Fig 8b). The ICAM-1 staining, although clearly visible, was surprisingly weak (Fig 8b). However, after hyaluronidase treatment the staining was dramatically increased (Fig 8c).

Discussion

The finding that monoclonal antibodies to rat ICAM-1 can inhibit the cell association of labeled HA to rat LEC in culture (Fig 6), provides further evidence that ICAM-1 is an important cell-surface receptor for HA. The inhibition

is only in the order of 50%, but as cell association was studied at 37°C the inhibition will not be complete due to the continuous appearance of unbound receptors from the interior of the cells. The affinity of high molecular weight HA for the receptors on LEC is very high and the labelled HA will probably compete well for binding to the free receptor sites. The possible existence of other HA binding sites on ICAM-1, not affected by 1A29 binding, also needs to be considered.

The monoclonal antibodies used in this study have previously been shown to cause inhibition of leukocyte adherence to endothelial cells. As the antibodies cause inhibition of HA binding to LEC, the HA binds to a site close to the leukocyte binding site on ICAM-1 and that binding of HA to endothelial cells causes inhibition of leukocyte adherence to the same cells. Systemic administration of HA can then have a beneficial effect on inflammatory conditions. Such effects, similar to the reduced inflammation seen with systemic treatments with antibodies to ICAM-1, have been described in animal models of acute and chronic inflammation.

The increased uptake in tumour tissue of the intravenously administered radiolabelled HA (Fig 7), indicates that binding structures for HA are present in the tumour. The staining for HA, using a highly specific biotinylated HA binding protein, indicated that the HA was localized in and around the vessels (Fig 8a). As expected, the staining for HARLEC/ICAM-1 was also found in vessels, but was rather weak (Fig 8b). Administration of HA to corneal EC causes a reduced immunohistochemical staining of CEC receptors which can be restored by hyaluronidase treatment, we also tried such hyaluronidase treatment of the tumour sections. The effect was a dramatic increase in HARLEC/ICAM-1 staining (Fig 8c), indicating that HA, bound to HARLEC/ICAM-1, caused an inhibition of the binding of the specific antibody.

Intravenously administered HA is predominantly bound to

endothelial cells via ICAM-1. This points to using HA as a carrier of cytotoxic drugs to tumours expressing this type of HA-receptor.

As many changes can be made to examples of the invention without departing from the scope of the invention, it is intended that all material contained herein be interpreted as illustrative of the invention and not in a limiting sense.